the highest versus the lowest quartiles for consumption of total fruit and vegetables, total vegetables and total fruit were 1.24 (95% CI 0.82–1.88; *P*-trend = 0.36), 1.34 (95% CI 0.87–2.07; *P*-trend = 0.17) and 1.52 (95% CI 0.91–2.52; *P*-trend = 0.17), respectively. Neither multivariate adjustments nor the exclusion of subjects with colon cancer diagnosed during the first three years of follow-up made any material changes to the findings.

We observed no associations between total fruit and vegetables, total vegetables and total fruit intakes and the risk of colon cancer when men and women were analysed separately. Specifically, the multivariate RRs for men in the highest versus the lowest quartiles for consumption of total fruit and vegetables, total vegetables and total fruit were 0.92 (95% CI 0.54–1.59; *P*-trend = 0.84), 1.00 (95% CI 0.56–1.77; *P*-trend = 0.91) and 1.75 (95% CI 0.89–3.44; *P*-trend = 0.23), respectively. Corresponding RRs for women were 1.55 (95% CI 0.72–3.32; *P*-trend = 0.36), 1.65 (95% CI 0.78–3.49; *P*-trend = 0.13) and 0.99 (95% CI 0.23–4.25; *P*-trend = 0.79).

Table 3 presents the relative risks for rectal cancer according to the total fruit and vegetables, total vegetables and total fruit consumption quartiles. After adjustment for age and sex, consumption of total fruit and vegetables, total vegetables and total fruit showed no association with the risk of rectal cancer. The age- and sex-adjusted RRs for the highest versus the lowest quartiles for consumption of total fruit and vegetables, total vegetables and total fruit were 1.12 (95% CI 0.68–1.84; *P*-trend = 0.40), 1.17 (95% CI 0.70–1.94; *P*-trend = 0.49) and 1.33 (95% CI 0.71–2.51; *P*-trend = 0.37), respectively. Again, neither multivariate adjustments nor the exclusion of subjects with rectal cancer diagnosed during the first three years of follow-up had any substantial impact on the findings.

As with colon cancer, we observed no association between total fruit and vegetables, total vegetables and total fruit consumption and the risk of rectal cancer when men and women were analysed separately. Specifically, multivariate RRs for men in the highest versus the lowest quartile for consumption of total fruit and vegetables, total vegetables and total fruit were 1.10 (95% CI 0.55–2.17; *P*-trend = 0.51), 1.32 (95% CI 0.67–2.60; *P*-trend = 0.23) and 0.28 (95% CI 0.04–2.09; *P*-trend = 0.75), respectively. Corresponding RRs for women were 1.26 (95% CI 0.56–2.86; *P*-trend = 0.42), 0.99 (95% CI 0.42–2.32; *P*-trend = 0.73). For total fruit consumption, there were no cases of rectal cancer in the reference category (the lowest quartile), so we conducted the analysis with reference to the lowest and second lower quartiles combined. The RR was 1.53 (95% CI 0.68–3.45; *P*-trend = 0.23).

Table 3 Relative risk (RR) (95% confidence interval) of rectal cancer for fruit and vegetables consumption

Food group and variable	Quartile				*P*-value for trend
	1 (lowest)*	2	3	4 (highest)	
Total fruit and vegetables					
Energy-adjusted consumption (g)	≤543	544–616	617–697	≥698	
Number of cases	30	19	28	33	
Person-years of follow-up	76 987	77 687	76 581	76 524	
Age- and sex-adjusted RR	1.00	0.66 (0.37–1.17)	1.00 (0.60–1.67)	1.12 (0.68–1.84)	0.40
Multivariate RR1	1.00	0.62 (0.35–1.12)	0.97 (0.57–1.65)	1.12 (0.67–1.89)	0.37
Multivariate RR2	1.00	0.57 (0.28–1.16)	1.00 (0.53–1.88)	1.08 (0.58–2.03)	0.47
Total vegetables					
Energy-adjusted consumption (g)	≤245	246–277	278–312	≥313	
Number of cases	27	26	26	33	
Person-years of follow-up	77 885	77 302	75 302	77 289	
Age- and sex-adjusted RR	1.00	0.92 (0.53–1.60)	1.02 (0.59–1.75)	1.17 (0.70–1.94)	0.49
Multivariate RR1	1.00	0.87 (0.50–1.52)	0.96 (0.55–1.66)	1.14 (0.67–1.93)	0.55
Multivariate RR2	1.00	1.05 (0.54 – 2.07)	1.13 (0.58 – 2.20)	1.18 (0.61–2.28)	0.61
Total fruit					
Energy-adjusted consumption (g)	≤95	96–169	170–241	≥242	
Number of cases	30	29	27	24	
Person-years of follow-up	76 662	76 854	77 861	76 402	
Age- and sex-adjusted RR	1.00	1.20 (0.71–2.03)	1.27 (0.72–2.26)	1.33 (0.71–2.51)	0.37
Multivariate RR1	1.00	1.26 (0.74–2.15)	1.36 (0.75–2.47)	1.41 (0.73–2.73)	0.30
Multivariate RR2	1.00	1.21 (0.63–2.30)	1.17 (0.56–2.44)	1.33 (0.60–2.86)	0.52

RR1 – multivariate relative risk with all cases of colon cancer in analysis; RR2 – multivariate relative risk with cases diagnosed in the first three years of follow-up excluded from analysis.
Total fruit and vegetables consist of the following: orange, other fruits and fresh fruit juices, green leaf vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles).
Total vegetables consist of the following: green leaf vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles).
Total fruit consist of the following: orange, other fruits and fresh fruit juices.
Multivariate models included: sex; age (in years); smoking status (never, past, currently smoking 1–19 cigarettes daily, currently smoking over 20 cigarettes daily); alcohol consumption (never, past, currently drinking 22.7 g or less alcohol daily, currently drinking 22.8 g or more alcohol daily); body mass index in $kg\,m^{-2}$ (18.4 or lower, 18.5–24.9, 25.0 or higher); education (up to 15 years of age, from 16 to 18 years, 19 years or older); family history of cancer (present, absent); walking time (less than 1 h daily, over 1 h daily); meat consumption (in quartiles – 45 g or less, 46–52 g, 53–62 g or 63 g or more daily).
*Referent category.

We further examined associations between the consumption of six specific types of vegetable (green leafy vegetables, carrots and pumpkins, tomatoes, cabbage and lettuce, Chinese cabbage and *tsukemono* (pickles)) and three types of fruit (oranges, other fruits and fresh fruit juices) and the risks of colon and rectal cancer, but found no significant correlations.

Discussion

In this population-based prospective cohort study in Japan, we found no significant associations between the intake of fruit or vegetables and the risk of colon or rectal cancer. Six prospective cohort studies published over the past 5 years have investigated the association between fruit and vegetable consumption and the risk of colon and rectal cancer. Of these, only one Swedish study found an inverse correlation[35]; the remaining five reported no association[30–34]. Our results are consistent with those of the latter five studies.

There are several possible interpretations of our finding that fruit and vegetable intake had no protective effect against colorectal cancer. First, the range of fruit and vegetable consumption among the subjects may not have been wide enough to produce positive effects. Among our study subjects, there was only a 1.5-fold difference across the total fruit and vegetables consumption quartiles (median intake was 506 g in the lowest and 758 g in the highest). Terry *et al.* reported an increased risk of colorectal cancer among the lowest consumers of total fruit and vegetables[35]. In their study, there was a 3.0-fold difference across the fruit and vegetable consumption quartiles. In contrast, although their study showed a wide range of consumption (a 3.9-fold difference across the quintiles for vegetables and a 10.0-fold difference for fruit), Flood *et al.* reported null association between the risks of colorectal cancer and fruit and vegetable consumption[30]. Thus, effect of range does not provide a full explanation for our null associations.

Second, we have to consider the effects of possible misclassification when completing the FFQ. If many FFQs containing misclassifications are used to estimate dietary consumption, the general results are likely to be distorted, and the effect of fruit and/or vegetables consumption on the risks of colorectal cancer risk might be attenuated. However, in our validation study, the Spearman correlation coefficients for fruit and vegetables consumption were relatively high, ranging from 0.50 to 0.59[38]. Thus, the effect of misclassification may not fully explain our null results.

In conclusion, during a population-based prospective cohort study in Japanese subjects, we found no association between the consumption of fruit and vegetables and the risks of colon and rectal cancers. These findings are in agreement with the results of several other similarly designed studies, and add to the debate regarding the suggested protective effects of fruit and vegetables against colorectal cancer.

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Original Article

Green Tea and the Risk of Colorectal Cancer: Pooled Analysis of Two Prospective Studies in Japan

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BACKGROUND: Although laboratory experiments suggest protective effects of green tea against colorectal cancer, few prospective cohort studies have been conducted.
METHODS: We conducted a pooled analysis of two prospective cohort studies among residents in Miyagi Prefecture in rural northern Japan. The first study started in 1984 and included 26,311 subjects. The second study started in 1990 and included 39,604 subjects. The subjects responded to a self-administered questionnaire including an item on green tea consumption. With 7 to 9 years of follow-up, 305 colon and 211 rectal cancers were identified in the two cohorts through record linkage to a regional cancer registry. We used Cox regression to estimate the hazard ratio (HR) of colorectal cancer according to the consumption of green tea with adjustment for potential confounders, and pooled the estimates obtained from each cohort by general variance-based method.
RESULTS: Multivariate pooled HRs for colon cancer associated with drinking 1-2, 3-4, and 5 or more cups of green tea per day, as compared with less than 1 cup per day, were 1.06 (95% confidence interval [CI] = 0.74-1.52), 1.10 (0.78-1.55), 0.97 (0.70-1.35), respectively (trend p = 0.81). Corresponding HRs for rectal cancer were 0.85 (95% CI = 0.56-1.29), 0.70 (0.45-1.08), 0.85 (0.58-1.23), respectively (trend p = 0.31).
CONCLUSIONS: Consumption of green tea was not associated with lower risk of colorectal cancer.
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Key words: Tea, Colorectal Neoplasms, Prospective Studies, Japan.

A potential anti-carcinogenic effect of green tea has drawn much interest in experimental and epidemiologic studies.[1] A fairly large number of epidemiologic studies have investigated the protective association between green tea and gastric cancer,[2-6] but a few studies have shown an inhibitory effect of green tea or tea catechin in the development of chemically-induced carcinoma of the colorectum as well as of the stomach. Sepcifically, in vitro experiments suggest that green tea polyphenols inhibit the formation of heterocyclic amines, which is formed during the cooking of meats and fish and is implicated in the development of colorectal cancer.[7] Animal experiments also suggest that green tea polyphenols suppress colon-carcinogenesis induced by heterocyclic amines.[8]

Epidemiologically, five case-control studies and one prospective cohort study have examined the association between consumption of green tea and the risk of colorectal cancer.[5, 9-13] For colon cancer, a case-control study in Japan found a significant inverse association,10 while the other five studies found no significant relation.[5, 9, 11-13] For rectal cancer, a case-control study in China found a significant inverse association,[12] while the other five studies found no significant relation.[5, 9-11, 13] The only prospective cohort study conducted among atomic bomb survivors of Hiroshima and Nagasaki, Japan, found no association between

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consumption of green tea and risk of colon or rectal cancer.[13]

To further examine the association between consumption of green tea and the risk of colorectal cancer, we conducted a pooled analysis of two population-based prospective cohort studies in Miyagi Prefecture in rural northern Japan.

METHODS

Study Cohort

Study designs of the two cohort studies have been described in detail elsewhere.[14, 15] Briefly, Cohort 1 started in January 1984, when we delivered a self-administered questionnaire to 33,453 men and women (40 years of age or older) in three municipalities of Miyagi Prefecture. Usable questionnaire were returned from 31,345 subjects (93.7%).[14] For Cohort 2, we delivered a self-administered questionnaire from June through August 1990 to 51,921 men and women (40-64 years of age) in 14 municipalities of the Prefecture. Usable questionnaires were returned from 47,605 subjects (91.7%).[15] Protocols for the two cohort studies were approved by the institutional review board of Tohoku University Graduate School of Medicine. We considered the return of the self-administered questionnaires signed by the subjects to imply their consent to participate in the studies.

Exposure Data

The questionnaires asked about "recent" (Cohort 1) or "usual" (Cohort 2) consumption of green tea. The two questionnaires used the same five categories: never, occasionally, 1-2 cups per day, 3-4 cups per day, 5 or more cups per day. "Never" and "occasionally" categories were collapsed into the single category "less than one cup per day" for the purpose of this analysis.

To examine the reproducibility and validity of the questionnaire measurements of green tea intake, we collected 12-day diet records from 56 men and 60 women who lived in the two municipalities in the study district.16 Spearman correlation coefficients between green tea consumption assessed by the Cohort 2 questionnaire and that measured by the diet records were 0.71 in men and 0.53 in women. Spearman correlation coefficients between green tea intake measured by the two questionnaires administered 12 month apart were 0.63 in men and 0.64 in women.

Follow-up

For both Cohorts, we used population registries of the municipalities to obtain information on the vital and residential status of the subjects. We ascertained the incidence of cancer using the Miyagi Prefectural Cancer Registry covering the study areas.[17] In this cancer registry, the proportion of colon cancer cases for which information was available only from death certificates was 3% in men and 7% in women, the proportion of rectal cancer cases for which information was available only from death certificates was 2% in men and 4% in women.[17] Follow-up was conducted from January 1, 1984, through December 31, 1992, for Cohort 1, and from August 1, 1990, through March 31, 1997, for Cohort 2. The proportions of subjects who were lost to follow-up were 18.5% for Cohort 1, and 3.9% for Cohort 2. We defined subjects who were lost to follow-up as those who moved out of the study areas during follow-up period, and we assumed that subjects who had not have died or lost to follow-up remain alive in the study areas. In analysis, lost to follow-up was treated as censoring case.

We excluded cancer cases prevalent at baseline (541 in Cohort 1, and 1,110 in Cohort 2). We also excluded the subjects who did not respond to the question on green tea (4,493 in Cohort 1, and 6,891 in Cohort 2). Consequently, our analysis included 26,311 subjects with 269 colorectal cancer cases (158 colon and 111 rectum) in Cohort 1, and 39,604 subjects with 247 with colorectal cancer cases (147 colon and 100 rectum) in Cohort 2.

Statistical Analysis

We counted person-year of follow-up for each subject from the beginning of follow-up until the date of diagnosis of colorectal cancer, the date of death, or the end of the follow-up period, whichever occurred first. Total person-years accrued were 200,039 for Cohort 1 and 290,836 for Cohort 2.

We estimated hazard ratios (HRs) and their 95% confidence intervals (CIs) of colon and rectal cancer according to the level of green tea consumption. We used Cox proportional-hazards regression to adjust for potentially confounding variables. For the two Cohorts, we considered the following variables as potential confounders: sex, age, family history of colorectal cancer, smoking status, body mass index (kg/m^2), alcohol consumption, and consumption frequencies of black tea and coffee. For Cohort 1, we further adjusted for consumption frequencies of meat, green or yellow vegetables, other vegetables and fruits. For Cohort 2, we further adjusted for consumption frequencies of beef, pork, ham, liver, spinach, carrot and pumpkin, tomato, orange, other fruits, and juice.

To obtain a summary measure of results from Cohort 1 and Cohort 2, the general variance-based method was used to combine each HR and 95% CI.[18] P values for the test of linear trend were calculated by treating the green tea consumption category as an ordinal variable. All reported P values are two-tailed.

RESULTS

Table 1 compares the characteristics between subjects who consumed less than one cup per day and those who consumed five or more cups of green tea per day. For both Cohorts, men who consumed five or more cups per day were older, more likely to be current smokers and to consume black tea, less likely to consume coffee, as compared with the men who consumed less than one cup per day.

In Cohort 1, women who consumed five or more cups per day were older, more likely to be current smokers, drinkers and to consume black tea, less likely to consume coffee, as compared with the women who consumed less than one cup per day. In Cohort 2, women who consumed five or more cups per day were

Table 1. Characteristics of the subjects according to green tea consumption.

	Men				Women			
	Cohort 1		Cohort 2		Cohort 1		Cohort 2	
	Green tea consumption (cups / day)				Green tea consumption (cups / day)			
	<1	5+	<1	5+	5+	5+	5+	5+
Characteristics								
No.	2,253	4,870	5,640	4,782	4,782	4,782	4,782	4,782
Age (yr), mean ± standard deviation	56.3 ± 11.3	57.6 ± 10.8	50.5 ± 7.5	53.7 ± 7.4	53.7 ± 7.4	53.7 ± 7.4	53.7 ± 7.4	53.7 ± 7.4
Smoking (%)								
Never	27.9	18.8	21.1	17.1	17.1	17.1	17.1	17.1
Past	23.8	23.2	20.2	20.8	20.8	20.8	20.8	20.8
Current (1-19 cigarettes / day)	18.9	17.2	16.2	13.5	13.5	13.5	13.5	13.5
Current (20+cigarettes / day)	29.4	40.9	42.6	48.7	48.7	48.7	48.7	48.7
Alcohol drinkig (%)								
Never	16.3	15.6	15.9	17.7	17.7	17.7	17.7	17.7
Past	10.6	8.0	7.2	8.0	8.0	8.0	8.0	8.0
Current	73.1	76.4	76.9	74.4	74.4	74.4	74.4	74.4
Body mass index (%)								
<18.5	5.2	4.4	1.9	2.2	2.2	2.2	2.2	2.2
18.5-24.9	72.3	73.4	70.5	68.5	68.5	68.5	68.5	68.5
25.0+	22.5	22.2	27.7	29.3	29.3	29.3	29.3	29.3
Daily beverage consumption (%)								
Black tea (cups / day)								
Never	65.0	47.6	64.9	59.8	59.8	59.8	59.8	59.8
Occasionally	31.3	44.8	33.2	36.8	36.8	36.8	36.8	36.8
1-2	2.9	5.6	1.5	2.3	2.3	2.3	2.3	2.3
3+	0.8	1.9	0.3	1.1	1.1	1.1	1.1	1.1
Coffee (cups / day)								
Never	34.7	18.0	20.6	19.8	19.8	19.8	19.8	19.8
Occasionally	35.2	46.5	32.9	41.7	41.7	41.7	41.7	41.7
1-2	19.5	25.2	29.0	24.6	24.6	24.6	24.6	24.6
3+	10.6	10.4	17.5	13.9	13.9	13.9	13.9	13.9

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) of colon and rectum cancer accoding to green-tea consumption.*

	Green-tea consumption (cups/day)				
	<1	1 or 2	3 or 4	5+	P for trend
			Colon		
No.of cases / person-year of follow-up					
Cohort 1	26/36,642	30/34,157	36/43,780	66/85,460	
Cohort 2	39/84,398	29/68,774	36/62,753	43/74,911	
Sex- and age-adjusted HR					
Cohort 1	1.0	1.25 (0.74-2.11)	1.13 (0.68-1.86)	1.02 (0.65-1.61)	0.79
Cohort 2	1.0	0.93 (0.58-1.51)	1.11 (0.71-1.75)	0.94 (0.61-1.45)	0.93
Pooled	1.0	1.06 (0.75-1.52)	1.12 (0.80-1.56)	0.98 (0.71-1.34)	0.80
Multivariate HR1					
Cohort 1	1.0	1.19 (0.70-2.03)	1.08 (0.65-1.80)	1.03 (0.65-1.64)	0.87
Cohort 2	1.0	0.96 (0.59-1.57)	1.12 (0.70-1.77)	0.93 (0.59-1.46)	0.85
Pooled	1.0	1.06 (0.74-1.52)	1.10 (0.78-1.55)	0.97 (0.70-1.35)	0.81
Multivariate HR2					
Cohort 1	1.0	1.16 (0.65-2.08)	0.88 (0.49-1.58)	0.82 (0.49-1.39)	0.27
Cohort 2	1.0	1.01 (0.57-1.78)	1.23 (0.72-2.11)	0.84 (0.48-1.45)	0.67
Pooled	1.0	1.08 (0.72-1.62)	1.05 (0.71-1.57)	0.83 (0.57-1.21)	0.27
			Rectum		
No.of cases / person-year of follow-up					
Cohort 1	18/36,642	20/34,157	22/43,780	51/85,460	
Cohort 2	38/84,398	20/68,774	15/62,753	27/74,911	
Sex- and age-adjusted HR					
Cohort 1	1.0	1.20 (0.63-2.27)	1.01 (0.54-1.88)	1.17 (0.68-2.00)	0.68
Cohort 2	1.0	0.65 (0.38-1.12)	0.48 (0.27-0.88)	0.64 (0.39-1.05)	0.05
Pooled	1.0	0.84 (0.56-1.29)	0.69 (0.45-1.06)	0.84 (0.58-1.21)	0.27
Multivariate HR1					
Cohort 1	1.0	1.34 (0.70-2.56)	1.14 (0.60-2.15)	1.34 (0.77-2.33)	0.40
Cohort 2	1.0	0.62 (0.36-1.06)	0.44 (0.24-0.82)	0.57 (0.34-0.95)	0.02
Pooled	1.0	0.85 (0.56-1.29)	0.70 (0.45-1.08)	0.85 (0.58-1.23)	0.31
Multivariate HR2					
Cohort 1	1.0	1.09 (0.49-2.41)	1.00 (0.46-2.15)	1.31 (0.68-2.51)	0.39
Cohort 2	1.0	0.54 (0.27-1.07)	0.39 (0.18-0.84)	0.66 (0.37-1.20)	0.14
Pooled	1.0	0.73 (0.43-1.22)	0.62 (0.36-1.07)	0.90 (0.58-1.40)	0.67

*: The multivariate hazard ratio (HR) has been adjusted for sex; age; family history of colorectal cancer; cigarette smoking; alcohol consumption; body mass index in kg / m^2 (<18.5, 18.5-24.9, 25.0+): consumption of black tea, and coffee. The multivariate HR for cohort1 has also been adjusted for consumption of meat, green-yellow vegetables, other vegetables, and fruits. The multivariate HR for Cohort2 has also been adjusted for consumption of beef, pork, ham, chicken, liver, spinach, carrot or pumpkin, tomato, orange, other fruits, and juice. HR1 denotes the relative risk with all cases of colorectal cancer included in the multivariate analysis, and HR2 the relative risk with cases diagnosed in the first three years of follow-up excluded from the analysis.
95% confidence intervals in parentheses.

Table 3. Pooled multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) of colorectal cancer according to green tea consumption.

			Green-tea consumption (cups/day)				
			<1	1 or 2	3 or 4	5+	P for trend
			Colon				
Sex*	Men	No. of cases	36	39	46	64	
		Pooled multivariate HR	1.0	1.32 (0.83-2.10)	1.35 (0.86-2.12)	1.12 (0.72-1.74)	0.69
	Women	No. of cases	29	20	26	45	
		Pooled multivariate HR	1.0	0.78 (0.43-1.40)	0.78 (0.45-1.35)	0.79 (0.49-1.29)	0.34
Alcohol consumption**	Current drinkers	No. of cases	33	37	38	52	
		Pooled multivariate HR	1.0	1.30 (0.80-2.10)	1.16 (0.72-1.87)	0.98 (0.61-1.57)	0.77
	Nondrinkers	No. of cases	26	16	23	42	
		Pooled multivariate HR	1.0	0.73 (0.39-1.37)	0.82 (0.46-1.46)	0.86 (0.52-1.44)	0.71
			Rectum				
Sex*	Men	No. of cases	37	27	19	36	
		Pooled multivariate HR	1.0	0.85 (0.50-1.45)	0.58 (0.32-1.04)	0.62 (0.38-1.02)	0.02
	Women	No. of cases	19	13	18	42	
		Pooled multivariate HR	1.0	0.81 (0.40-1.66)	0.95 (0.48-1.89)	1.30 (0.70-2.42)	0.23
Alcohol consumption**	Current drinkers	No. of cases	32	24	16	34	
		Pooled multivariate HR	1.0	0.85 (0.49-1.49)	0.52 (0.28-0.97)	0.64 (0.38-1.09)	0.05
	Nondrinkers	No. of cases	15	13	14	30	
		Pooled multivariate HR	1.0	0.96 (0.42-2.22)	0.87 (0.36-2.10)	1.17 (0.56-2.42)	0.54

*: The pooled multivariate hazard ratio (HR) has been adjusted for age; family history of colorectal cancer; cigarette smoking; alcohol consumption; body mass index in kg / m^2 (<18.5, 18.5-24.9, 25.0+); consumption of black tea, and coffee.

**: The pooled multivariate hazard ratio (HR) has been adjusted for sex; age; family history of colorectal cancer; cigarette smoking; body mass index in kg / m^2 (<18.5, 18.5-24.9, 25.0+); consumption of black tea, and coffee.

The multivariate HR for cohort1 has also been adjusted for consumption of meat, green-yellow vegetables, other vegetables, and fruits.

The multivariate HR for cohort2 has also been adjusted for consumption of beef, pork, ham, chicken, liver, spinach, carrot or pumpkin, tomato, orange, other fruits, and juice.

95% confidence intervals in parentheses.

older, more likely to be current smokers and to consume black tea, less likely to be current alcohol drinkers and to consume coffee, as compared with the women who consumed less than one cup per day.

Table 2 presents the HRs for colon and rectal cancer according to green tea consumption. After adjustment for sex and age, green tea consumption was not associated with the risk of colon cancer in Cohort 1, Cohort 2, and the two Cohorts combined. Multivariate adjustment or the exclusion of cases of colon cancer diagnosed in the first three years of follow-up did not change the findings materially.

After adjustment for sex and age, green tea consumption was not associated with risk of rectal cancer in Cohort 1, but was associated with lower risk of rectal cancer in Cohort 2. When the two Cohorts were pooled, however, green tea consumption was not associated with risk of rectal cancer. Null results in the pooled analysis did not change materially with multivariate adjustment or with the exclusion of cases of rectal cancer diagnosed in the first three years of follow-up.

We conducted stratified analyses according to sex, age, cigarette smoking, alcohol consumption, body mass index, and family history of colorectal cancer. Table 3 presents the pooled HRs stratified by sex and alcohol consumption. For colon cancer, we found no different results by sex and alcohol consumption. For rectal cancer, green tea consumption was associated with a decreased risk among men and current drinkers, but not among women and nondrinkers. We did not observe different findings for other variables.

DISCUSSION

In this pooled analysis of two population-based, prospective cohort studies in rural northern Japan, we generally found no association between green tea consumption and the risk of colorectal cancer. Green tea intake was associated with lower risk of rectal cancer among men and alcohol drinkers, but not among women and nondrinkers. Green tea was not related with lower risk of colon cancer irrespective of subjects' sex or drinking status.

Five case-control studies and 1 prospective cohort study have examined the association between green tea and risk of colorectal cancer.[5, 9-13] For colon cancer, a case-control study in Japan found a significant inverse association,10 while other studies found no significant relation.[5, 9-11, 13] For rectal cancer, a case-control study in China found a significant inverse association,[12] while other studies found no significant relation.[5, 9-11, 13] The inverse association observed in the two case-control studies may be partly due to recall bias.

Our study had several methodological advantages over prior studies of green tea and the risk of colorectal cancer, which include the use of prospective design, the use of validated food frequency questionnaire, and the large number of colorectal cancer cases accrued.

We observed significantly lower risk of rectal cancer associated with higher green tea intake among Cohort 2, men and alcohol drinkers, but not among Cohort 1, women or nondrinkers. Pooling of the results from the two Cohorts may require caution in interpretation, because a statistical test for the heterogeneity of the two Cohort results was not significant for sex- and age-adjusted HRs ($p>0.05$) but for multivariate HRs ($p<0.05$). Although it is possible that green tea is protective against rectal cancer only among these subgroups of subjects, chance may be a more likely explanation for the discrepancies according to the subgroups.

Although all of the six previous studies of green tea and colorectal cancer included both men and women,[5, 9-13] only one case-control study in China[12] reported sex-specific results: green tea intake was significantly associated with lower risk of rectal cancer in men and women, while it was not significantly associated with risk of colon cancer in men or women. None of the previous studies reported the results of analyses stratified by categories of alcohol consumption. Future studies of green tea and colorectal cancer should report the findings stratified by sex and drinking status.

We used self-reports for a measure of green-tea consumption. In a validation study of the food frequency questionnaire in which 119 subjects provided four 3-day food records in one year and then responded to the questionnaire, we observed a reasonably high degree of validity and reproducibility for the questionnaire measurement of green tea intake; Spearman's correlation coefficient for green-tea intake measured by the questionnaire and by the food records was 0.66, and the correlation between the two questionnaires administered in 6-month interval was 0.66.[14]

The proportion of subjects who were lost to follow-up was 18.5% for Cohort 1. The subjects who were lost to follow-up were more likely to be young, be current smokers, have family history of colorectal cancer, and less likely to be current alcohol drinkers, obese, as compared with those who could be follow-up.

As a potential limitation of the study, we could not specifically examine the effect of very high consumption of green tea, since the highest category in our questionnaire was five or more cups per day and we could not divide this category into subcategories (such as 5-9 cups and 10 or more cups). However, the validation study of our food frequency questionnaire found that 53% of the subjects who claimed to consume five or more cups per day in the questionnaire actually consumed seven or more cups per day according to 12-day diet records. This result suggests that a substantial proportion of our subjects in the highest consumption category actually consumed very high amounts of green tea. It is therefore unlikely that we failed to detect a large decrease in the risk of colorectal cancer associated with very high consumption of green tea.

We observed lower consumption of green tea in Cohort 2 than in Cohort 1 subjects. The most likely explanation would be the actual difference in green-tea intake between the two cohorts. Different validity of the questionnaire among the two cohorts may be another possibility, but it is unlikely that the different validity

of the questionnaire lead to the different observations only for rectal cancer (inverse association in Cohort 2 and no association in Cohort 1) but not for colon cancer (no association in both Cohorts).

In conclusion, our pooled analysis of two population-based prospective cohort studies conducted in rural Japan showed no overall association between the consumption of green tea and the risk of colorectal cancer. The inverse association for rectal cancer observed in subgroup analyses (Cohort 1, men, and current drinkers) warrants further investigations.

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Coffee consumption and the risk of primary liver cancer: Pooled analysis of two prospective studies in Japan

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Although case-control studies suggested that coffee consumption is associated with a decreased risk of liver cancer, no prospective cohort study has been carried out. To examine the association between coffee consumption and the risk of liver cancer, we conducted a pooled analysis of data available from 2 cohort studies in Japan. A self-administered questionnaire about the frequency of coffee consumption and other health habits was distributed to 22,404 subjects (10,588 men and 11,816 women) in Cohort 1 and 38,703 subjects (18,869 men and 19,834 women) in Cohort 2, aged 40 years or more, with no previous history of cancer. We identified 70 and 47 cases of liver cancer among the subjects in Cohort 1 (9 years of follow-up with 170,640 person-years) and Cohort 2 (7 years of follow-up with 284,948 person-years), respectively. We used Cox proportional hazards regression analysis to estimate the relative risk (RR) and 95% confidence interval (CI) of liver cancer incidence. After adjustment for potential confounders, the pooled RR (95% CI) of drinking coffee never, occasionally and 1 or more cups/day were 1.00 (Reference), 0.71 (0.46–1.09) and 0.58 (0.36–0.96), respectively (p for trend = 0.024). In the subgroup of subjects with a history of liver disease, we found a significant inverse association between coffee consumption and the risk of liver cancer. Our findings support the hypothesis that coffee consumption decreases the risk of liver cancer. Further studies to investigate the role of coffee in prevention of liver cancer among the high-risk population are needed.
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Key words: coffee; liver neoplasms; incidence; prospective studies; Japan

Primary liver cancer is the third most common cause of death from cancer worldwide.[1] The incidence of liver cancer is highest in Eastern Asia, including Japan.[2] Although its incidence is lower in Europe[3,4] and the United States,[5] it has been increasing over the last few decades.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are established causes of liver cancer,[6] and 59.6% and 23.6% of liver cancers worldwide are considered attributable to HBV and HCV, respectively.[7] Epidemiologic studies have indicated that alcohol drinking[8,9] and tobacco smoking[10] are also associated with an increased risk of liver cancer.

There are several sets of data supporting the possibility that coffee consumption has a preventive effect against liver cancer. Animal experiments have indicated that coffee has inhibitory effects against chemical carcinogenesis in liver tissue.[11] Furthermore, epidemiologic studies have demonstrated that coffee consumption is inversely related to serum liver enzyme activity,[12–15] and has an inverse association with the incidence of liver cirrhosis.[16,17] Recent case-control studies in Italy and Greece have suggested that coffee consumption is associated with a decreased risk of liver cancer.[18–20]

All the existing epidemiologic evidence related to coffee consumption and liver cancer has been derived only from case-control studies.[18–20] To further clarify the association between coffee consumption and the risk of liver cancer, a prospective cohort study is essential. Our present study was conducted to examine the association between coffee consumption and the risk of primary liver cancer based on population-based prospective cohort studies in Japan.

Material and methods

Study cohorts

Our present study was based on a pooled analysis of 2 prospective cohort studies in Japan. The study designs for the 2 studies have been described in detail elsewhere.[21–23] Briefly, for Cohort 1, we delivered a self-administered questionnaire in January 1984 to 33,453 residents, 40 years of age or older, in 3 municipalities of Miyagi Prefecture. Usable questionnaires were returned from 31,345 (93.7%) of the subjects. For Cohort 2, we delivered a self-administered questionnaire between June–August 1990 to 51,921 residents, 40–64 years of age, in 14 municipalities of Miyagi Prefecture. Usable questionnaires were returned from 47,605 (91.7%) of the subjects. Study protocols for the 2 cohorts were approved by the institutional review board of Tohoku University Graduate School of Medicine. We considered that the return of the self-administered questionnaires signed by the subjects implied their consent to participate in the study.

Exposure data

In both cohorts, the questionnaire included items inquiring about the frequency of recent consumption of 3 kinds of beverages (coffee, green tea, black tea) and food items, as well as questions on smoking status and history of disease. In the question about history of liver disease, the subjects were simply asked, "Have you had any liver disease?" Thus, we did not ascertain the name of the liver disease. Alcohol consumption was assessed by asking if the subject had never drunk, or was a former, or current, drinker. Current drinkers were also asked about their frequency of drinking and the amount of alcohol consumed on one occasion.

We asked the subjects about their frequency of coffee consumption according to 5 categories: never, occasionally, 1–2 cups per day, 3–4 cups per day and 5 or more cups per day. No question about the method used to brew the coffee was asked. The volume of a typical cup of coffee was 150 ml in the study region. The validation study of beverage consumption indicated that the self-reported frequency of coffee consumption among the subjects was satisfactorily valid and reliable. One hundred thirteen subjects in the study population responded to the questionnaire twice, 1 year apart, and provided four 3-day diet records within the year. Spearman's coefficient for the correlation between the amounts of coffee consumed according to the questionnaire and the amounts

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TABLE I – CHARACTERISTICS OF THE SUBJECTS ACCORDING TO COFFEE CONSUMPTION[1]

	Coffee consumption (cups/day)					
	Cohort 1			Cohort 2		
	Never	Occasionally	≥1	Never	Occasionally	≥1
No. of subjects	4938	9507	7959	6954	14130	17619
Age (years), mean ± SD[2]	60.4 ± 11.8	56.0 ± 10.5	52.4 ± 9.7	54.2 ± 7.0	52.8 ± 7.2	48.9 ± 7.1
History of liver disease (%)	5.4	4.7	4.5	6.6	4.8	4.4
Male (%)	41.0	46.1	52.5	47.1	46.8	51.0
Alcohol drinking (%)						
Never	48.9	38.3	33.4	45.9	43.7	39.0
Formerly	7.7	5.0	5.1	6.6	5.1	5.4
Occasionally	21.0	32.6	34.6	18.4	24.8	28.9
Daily, <45.6 g/d	7.0	8.5	9.4	8.0	8.3	9.2
Daily, ≥45.6 g/d	15.4	15.7	17.4	21.1	18.2	17.6
Smoking (%)						
Never	62.4	60.6	47.2	56.9	55.8	47.3
Formerly	13.8	12.4	13.4	14.0	12.7	10.2
Daily, <19 cigarettes	10.6	10.7	12.7	10.5	10.7	11.0
Daily, ≥20 cigarettes	13.2	16.3	27.7	18.6	20.8	31.7
Daily consumption (%)						
Green tea (≥3 cups/day)	57.0	68.6	60.3	47.9	52.2	39.6
Black tea (≥3 cups/day)	0.7	0.9	2.7	0.3	0.4	0.8

[1]$n = 61, 107$.–[2]SD denotes standard deviation.

consumed according to the diet records was 0.70, and the correlation between consumption measured by the 2 questionnaires over 1 year was 0.72.

Follow-up

The end point in our analysis was incidence of primary liver cancer defined as the topography code C22.0 and fifth digit behavior code for neoplasms/3 according to the International Classification of Diseases for Oncology (2nd Ed.; ICD-O-2).[24]

For both cohorts, we followed the vital and residential status of each subject using a population registry for each municipality. We ascertained the incidence of cancer using the Miyagi Prefectural Cancer Registry, one of the earliest and most accurate population-based cancer registries in Japan.[25] In this registry, the relevant cases were abstracted from medical records of hospitals by a medical doctor or trained medical record reviewer, except for the cases reported from an institution to the registry. A follow-up was conducted from 1 January 1984–31 December 1992 for Cohort 1, and from 1 August 1990–31 March 1997 for Cohort 2.

We excluded cancer cases prevalent at the baseline (541 cases in Cohort 1 and 1,110 cases in Cohort 2). Then, we excluded subjects who did not answer the question about coffee consumption (8,400 subjects in Cohort 1 and 7,792 subjects in Cohort 2). Consequently, our analysis included 22,404 subjects (10,588 men and 11,816 women) including a total of 70 cases of liver cancer (50 men and 20 women) in Cohort 1, and 38,703 subjects (18,869 men and 19,834 women) including a total of 47 cases of liver cancer (41 men and 6 women) in Cohort 2.

Diagnosis of the 117 primary liver cancer cases was confirmed by medical records ($n = 90$, 76.9%) or death certificates alone ($n = 27$, 23.1%). In the 90 cases of primary liver cancer reviewed from medical records, the diagnosis was confirmed by histologic or cytologic examination in 43 cases, and by imaging (ultrasonography, computed tomography, magnetic resonance imaging, or angiography) alone in 35 cases. Although medical records had been reviewed, no further information on the basis of diagnosis was obtainable from the registered data in 12 cases. Among the 43 cases of primary liver cancer established by histologic or cytologic examination, the histological types were hepatocellular carcinoma (ICD-O-2 morphology code M-8170/3, $n = 35$), unspecified cancer (M-8000/3, $n = 6$), adenocarcinoma (M-8140/3, $n = 1$) and hemangiosarcoma (M-9120/3, $n = 1$).

Statistical analysis

We counted the number of person-years of follow-up for each subject from the beginning of follow-up until the date of diagnosis of liver cancer, the date of emigration from the study districts, the date of death or the end of follow-up, whichever occurred first. Total person-years accrued were 170,640 for Cohort 1 and 284,948 for Cohort 2. We combined the upper 3 categories of coffee consumption into the single category "1 or more cups/day" because of the small number of subjects in each category. In fact, the numbers of patients with liver cancer who reported drinking coffee 1–2, 3–4 or 5 or more cups/day were 19, 11 and 0, respectively. Relative risk was computed as the incidence rate among subjects in each category of coffee consumption divided by the rate among those who had never drunk coffee. We considered subjects who had never drunk coffee as the reference group.

We used Cox proportional hazards regression analysis to estimate the relative risk (RR) and 95% confidence interval (CI) of liver cancer incidence according to categories of coffee consumption and to adjust for potentially confounding variables, using SAS version 8.2 statistical software (SAS Inc., Cary, NC).

As the primary outcome, we examined the association between coffee consumption and the risk of incidence of primary liver cancer. We considered the following variables to be potential confounders: age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], daily drinker who consumed <45.6 g alcohol/day, or 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day).

To obtain a summary measure of the results from Cohort 1 and Cohort 2, we used the general variance-based method.[26] The p-values for the analysis of linear trends were calculated by treating the coffee consumption category as an ordinal variable. All reported p-values are 2-tailed, and differences at $p < 0.05$ were considered statistically significant.

Results

Table I compares the characteristics of subjects according to coffee consumption. The subjects with higher coffee consumption tended to be younger and male, and were more likely to be drinkers and heavy smokers (20 cigarettes or more/day) and were less likely to have a history of liver disease. The consumption of

TABLE II – RELATIVE RISK (RR) AND 95% CONFIDENCE INTERVAL (CI) OF LIVER CANCER ACCORDING TO COFFEE CONSUMPTION

Variable	Coffee consumption (cups/day)			*p* for Trend
	Never	Occasionally	≥1	
No. of cases of liver cancer/person-years				
Cohort 1	29/36,988	25/74,226	16/59,427	
Cohort 2	12/51,017	21/104,459	14/129,471	
Age, gender-adjusted RR (95% CI)				
Cohort 1	1.00	0.48 (0.28–0.82)	0.44 (0.24–0.83)	0.0076
Cohort 2	1.00	0.92 (0.45–1.87)	0.61 (0.28–1.33)	0.19
Pooled	1.00	0.61 (0.40–0.94)	0.50 (0.31–0.82)	0.0041
Multivariate RR[1] (95% CI)				
Cohort 1	1.00	0.56 (0.33–0.97)	0.53 (0.28–1.00)	0.038
Cohort 2	1.00	1.05 (0.52–2.16)	0.68 (0.31–1.51)	0.30
Pooled	1.00	0.71 (0.46–1.09)	0.58 (0.36–0.96)	0.024

[1]Multivariate RR was adjusted for age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], drinker who consumed less than 45.6 g alcohol/day, 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day).

green tea did not vary according to the consumption of coffee. We observed a similar tendency in Cohort 2.

Table II shows the association between coffee consumption and the risk of primary liver cancer. We found that higher coffee consumption was significantly associated with a lower risk of incidence of liver cancer. The pooled multivariate RR (95% CI) of liver cancer in subjects who drank coffee never, occasionally, and 1 or more cups/day were 1.00, 0.71 (0.46–1.09) and 0.58 (0.36–0.96), respectively (p for trend = 0.024). In the analysis of each cohort, a similar trend was observed. Results remained essentially the same when we excluded the 41 cases (22 cases [13 men and 9 women] in Cohort 1, 19 cases [17 men and 2 women] in Cohort 2) with liver cancer diagnosed in the first 3 years of follow-up (data not shown).

When we did not count 27 cases confirmed by death certificate only (DCO) as primary liver cancer, the point estimate of the RR of liver cancer had a similar trend. The pooled multivariate RR of liver cancer in subjects who drank coffee never, occasionally, and 1 or more cups/day were 1.00, 0.87 (0.52–1.45) and 0.73 (0.41–1.29), respectively (p for trend = 0.25).

Table III shows the association between coffee consumption and the risk of liver cancer in subgroup analyses. The RR of liver cancer were below unity irrespective of whether the subjects were younger or older, male or female, current drinkers or not, current smokers or not and had had liver disease or not. A significant inverse association between coffee consumption and the risk of liver cancer was observed in the subjects with a history of liver disease (p for trend = 0.047), whereas the association was not significant in the subjects without a history of liver disease.

We also examined the relationship between green tea consumption and the risk of primary liver cancer, but the relationship was null. After adjustment for the same covariates as those used for analysis of coffee consumption, the pooled RR (95% CI) of primary liver cancer in subjects who drank 2 or less, 3–4, and 5 or more cups of green tea/day were 1.00, 1.20 (0.75–1.94) and 0.90 (0.56–1.44), respectively (p for trend = 0.70). We were unable to estimate the relationship between consumption of black tea and liver cancer incidence because the proportion of subjects who drank 1 or more cups of black tea/day was only 7.8% in Cohort 1 and 2.8% in Cohort 2.

Discussion

In this pooled analysis of 2 prospective cohorts, we found a statistically significant inverse association between coffee consumption and the incidence risk of primary liver cancer. This result is consistent with recent case-control studies in Italy and Greece.[20] Consumption of coffee in our subjects was not particularly low in comparison with the Western population. The proportion of subjects who reported drinking 1 or more cups of coffee/day was 41.9% in our study and 58.5% in the United States.[16] Almost half of the liver cancer cases occurred in the 2,985 subjects who reported a history of liver disease at the baseline. Although we had no specific information on liver diseases, this result was consistent with the strong association between chronic liver diseases such as chronic hepatitis or liver cirrhosis and the risk of liver cancer.[27]

Our study had several strengths. We recruited our subjects from the general population and identified a large number of cases of liver cancer among them. The information on coffee consumption and other variables was obtained before the cases of liver cancer were diagnosed, thus avoiding any effect of recall bias. The questionnaire used for measuring coffee consumption had a reasonably high level of validity and reproducibility. In addition, the inverse association between coffee consumption and the risk of liver cancer was unchanged after adjustment for, and stratification by, potential confounders. Moreover, to avoid any potential bias from subclinical conditions, we excluded subjects in whom liver cancer was diagnosed in the first 3 years of follow-up. The inverse association was unchanged after this exclusion.

Our study also had some limitations. First, we had no information about history of HBV or HCV infection. The prevalence of hepatitis B surface antigen (HBsAg) and antibodies against HCV (anti-HCV) among subjects 40 years of age or older in this area was 1.87% and 2.19%, respectively.[28] In Japan, 28% and 43% of liver cancers are estimated to be attributable to HBV and HCV, respectively.[7] If these viral infections were related to change in coffee consumption, the association between coffee consumption and the risk of liver cancer would be confounded. In our study, the RR of liver cancer were below unity, irrespective of whether the subjects had liver disease or not. In a previous study,[17] the inverse relationship between coffee consumption and the odds ratio of liver cirrhosis was independent of HCV and HBV infection. Because of the strong association between these virus infections and the risk of liver cancer, however, even a weak inverse association between these viral infections and coffee consumption could introduce negative confounding, which would lead to overestimation of the effect of coffee consumption on the decreased liver cancer risk. Measurement of HBV and HCV infections would be needed in further prospective studies.

Second, primary liver cancer cases identified on the basis of death certificates alone without confirmation by medical records might have a possibility of misclassifying secondary metastasis to the liver as primary liver cancer. We carried out an additional analysis not considering the DCO cases as primary liver cancer. The inverse association between coffee consumption and the risk of primary liver cancer was not materially changed. We believe it

TABLE III – POOLED MULTIVARIATE RELATIVE RISK (RR) AND 95% CONFIDENCE INTERVAL (CI) OF LIVER CANCER ACCORDING TO COFFEE CONSUMPTION BY VARIOUS SUBGROUPS

	Coffee consumption (cups/day)			p for Trend[1]
	Never	Occasionally	≥1	
Age				
40-59 (n = 46,718)				
No. of cases	14	23	20	
Multivariate RR[1] (95% CI)	1.00	0.88 (0.44–1.74)	0.81 (0.40–1.64)	0.59
60- (n = 14,389)				
No. of cases	27	23	10	
Multivariate RR[1] (95% CI)	1.00	0.58 (0.32–1.09)	0.44 (0.21–0.93)	0.015
Gender				
Male (n = 29,457)				
No. of cases	28	36	27	
Multivariate RR[1] (95% CI)	1.00	0.73 (0.44–1.21)	0.64 (0.37–1.12)	0.11
Female (n = 31,650)				
No. of cases	13	10	3	
Multivariate RR[1] (95% CI)	1.00	0.66 (0.28–1.57)	0.54 (0.14–2.07)	0.12
Alcohol drinking				
Never (n = 21,914)				
No. of cases	14	10	4	
Multivariate RR[1] (95% CI)	1.00	0.69 (0.29–1.65)	0.46 (0.14–1.52)	0.095
Former (n = 2,974)				
No. of cases	8	6	6	
Multivariate RR[1] (95% CI)	1.00	0.60 (0.20–1.78)	0.74 (0.23–2.39)	0.58
Current (n = 28,750)				
No. of cases	15	28	14	
Multivariate RR[1] (95% CI)	1.00	0.90 (0.47–1.71)	0.56 (0.24–1.29)	0.097
Smoking				
Never (n = 27,233)				
No. of cases	12	14	2	
Multivariate RR[1] (95% CI)	1.00	0.90 (0.38–2.11)	0.27 (0.06–1.32)	0.10
Former (n = 6,164)				
No. of cases	14	9	2	
Multivariate RR[1] (95% CI)	1.00	0.53 (0.21–1.34)	0.18 (0.04–0.84)	0.012
Current (n = 18,334)				
No. of cases	11	16	19	
Multivariate RR[1] (95% CI)	1.00	0.80 (0.36–1.75)	0.90 (0.41–1.97)	0.84
History of liver disease				
yes (n = 2,985)				
No. of cases	23	17	13	
Multivariate RR[1] (95% CI)	1.00	0.51 (0.27–0.97)	0.52 (0.25–1.07)	0.047
no (n = 58,122)				
No. of cases	18	29	17	
Multivariate RR[1] (95% CI)	1.00	0.98 (0.53–1.80)	0.75 (0.37–1.50)	0.33

[1]Multivariate RR was adjusted for age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], daily drinker who consumed less than 45.6 g alcohol/day, 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day). Each model stratified by gender, alcohol consumption, smoking status and history of liver disease did not include variables for each stratum, respectively.

is unlikely that the DCO cases distorted the inverse association substantially.

Third, we excluded 16,192 subjects because they did not answer the question on coffee consumption. Fifty-one cases of liver cancer were diagnosed in this group. We considered that the characteristics of subjects who did not report their coffee consumption were essentially similar to those of subjects who did. The 2 groups were similar with respect to the prevalence of current smokers (35.5% and 35.4% of the groups, respectively), current alcohol drinkers (51.4% and 54.2%, respectively), and a history of liver disease (4.2% and 4.9%, respectively), apart from the distribution of age classes (subjects 40–59 years of age made up 53.6% and 76.5% of the groups, respectively) and gender (men made up 41.3% and 48.2%, respectively). The pooled multivariate RR (95% CI) of liver cancer in the subjects who did not answer the question about their coffee consumption, as compared to those who did, was 1.23 (0.87–1.74). Thus, our result might not have been substantially biased by exclusion of the subjects who did not answer the question on coffee consumption.

Fourth, we were unable to distinguish between never and former coffee drinkers, as this information was not collected at the baseline. Such information would allow more precise estimation of the effects of coffee on liver cancer in further studies. Finally, we did not investigate the method used for brewing coffee. For practical purposes, however, we can consider that most of the subjects would have consumed instant or filtered coffee because unfiltered coffee is rarely consumed in Japan.[29]

Among the subjects with a history of liver disease, we observed a significant inverse relationship between coffee consumption and liver cancer. We speculate that coffee may prevent liver cancer more effectively among subjects with liver disease than among those without liver disease. If the subjects with a history of liver disease had reduced their coffee consumption at the time of baseline data collection because of ill health, an inverse association between coffee consumption and liver cancer would have been observed. Among the subjects with a history of liver disease, we did not observe a decreasing trend in the proportion of former alcohol drinkers and former smokers, according to the frequency of coffee consumption, who might have quit drinking and smoking due to ill health. In our data, among the subjects with a history of liver disease, the proportions of former alcohol drinkers who drank coffee never, occasionally or 1 or more cups/day were

13.5%, 11.5% and 13.1% respectively, and the corresponding proportions of former smokers were 17.7%, 19.3% and 17.6%, respectively. Kuper *et al.*[19] failed to estimate the odds ratio of liver cancer for coffee drinkers among a subgroup of subjects with HBsAg or anti-HCV because there were no controls who did not drink coffee among these subjects. Further studies to elucidate the preventive effects of coffee consumption against liver cancer among subjects with chronic hepatitis or liver cirrhosis are needed.

Meanwhile, among the subjects without a history of liver disease, the inverse association between coffee consumption and the risk of liver cancer was not significant, but the RR of liver cancer was below unity. Among the subjects without a history of liver disease, we could not conclude from our data whether we might fail to detect a significant inverse association between coffee consumption and the risk of liver cancer due to insufficient statistical power or whether there might be no association.

It remains unclear which ingredient(s) of coffee is protective against liver cancer. Mutagenic and antimutagenic effects of coffee and caffeine on cultured cells of bacterial and mammalian origin have been demonstrated, but mutagenic effects would be almost non-existent at the usual levels of coffee consumption in humans.[30] The caffeine concentration in coffee and green tea is 0.06% and 0.02%, respectively.[31] Caffeine might not have a protective effect against liver cancer because our study indicated that consumption of green tea was not associated with the risk of liver cancer. Coffee also contains chlorogenic acid, a phenolic compound, whose inhibitory effects on chemical carcinogenesis in the liver have been demonstrated in an animal model.[11] The diterpenes cafestol and kahweol, both present in coffee, have been implicated in anticarcinogenic activity,[32] but it seems unlikely that they would have had a protective effect against liver cancer in this study group because their quantity is almost negligible in instant and filtered coffee.[33]

In conclusion, we have found that coffee consumption is significantly associated with a decreased incidence of liver cancer. In addition, subgroup analysis among our subjects with a history of liver disease showed an inverse association between coffee consumption and the risk of liver cancer. Further studies to clarify the role of coffee in prevention of liver cancer among the population at high risk are needed.

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Short Communication

Association of Vegetable Intake with Urinary 6-Sulfatoxymelatonin Level

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Abstract

Melatonin is present in plants consumed as vegetables; however, only a limited number of vegetables have been tested for melatonin. The antiproliferative, antioxidative, and immunostimulatory effects of melatonin have been reported from laboratory studies. The potential protective effects of vegetable against cancer and cardiovascular disease may be partially attributable to an increased melatonin intake from vegetables. As a first step to test this hypothesis, we evaluated whether vegetable intake is associated with an increased urinary melatonin in 289 community-dwelling Japanese women. Diet, including vegetable consumption, was assessed with a validated 169-item semiquantitative food-frequency questionnaire. Urinary 6-sulfatoxymelatonin (aMT6-s) was measured in the first-void morning urines. There was a significant positive association between vegetable intake and urinary aMT6-s levels. The mean urinary aMT6-s was 16% higher in women with the highest quartile of vegetable intake than it was in those with the lowest quartile of intake. This association may be explained by the melatonin contained in vegetables. However, data should be regarded as preliminary because it is impossible to estimate dietary melatonin intake from vegetables and or from the entire diet because of incomplete data for melatonin in plants. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1333–5)

Introduction

Epidemiologic evidence has suggested that a high consumption of vegetables is associated with reduced risks of cancer and cardiovascular disease (1). The hypothesis that the antioxidant activity of vegetables contributes to their beneficial effects has been advanced (2). Several mechanisms, including vitamins, carotenoids, and flavonoids, are probably involved in these processes. Beyond these recognized constituents of vegetables, certain other substances may confer additional health benefits. In recent years, melatonin has been discovered in edible plants. To date, only a small number of plants have been tested for melatonin (3), but the results of the studies investigating the melatonin contents in plants suggest that melatonin is present in a wide number of plants (4), including commonly consumed vegetables, such as cabbages and white radish sprouts, which are with relatively high amounts of melatonin (3). Normally, melatonin production occurs during the dark phase of the day. The distinct diurnal variation has suggested the possibility of a regulatory function of melatonin in day/night-dependent physiologic processes, such as sleep (5). Recently, laboratory studies have also shown the antiproliferative, antioxidative, and immunostimulatory actions of melatonin (6). The protective effects of vegetable intake against cancer and cardiovascular disease may be partially attributable to an increase in melatonin intake resulting from the consumption of vegetables. As a first step to test this hypothesis, we evaluated whether vegetable intake is associated with an increased urinary melatonin in generally healthy women.

Materials and Methods

Study subjects were recruited among participants of a breast cancer screening at a general hospital in Gifu, Japan, between June and December, 2000. The hospital has been conducting mass screening campaign for breast cancer since the early 1980s. Municipal letters of invitation to the screening are mailed to women residing in its surroundings. A total of 432 women who were free of breast cancer participated in the present study (response rate was 68.5%). Informed consent was obtained from each woman. The study was approved by the institutional review board.

Each woman responded to a self-administered questionnaire asking basic demographic characteristics, diet, exercise, smoking and drinking habits, and medical and reproductive histories. A nurse epidemiologist visited participants and collected first-void morning urines on the next morning. The blood and urine samples were frozen and stored at −80°C until assayed.

Intakes of vegetables and other foods and nutrients were estimated using a 169-item semiquantitative food-frequency questionnaire. Detailed information on the questionnaire, including its validity and reproducibility, has been described elsewhere (7, 8). For example, the Spearman correlation coefficients between this questionnaire and 12 daily diet records kept over a 1-year period for total energy, fat, protein, carbohydrates, and vegetables were 0.53, 0.52, 0.63, 0.53, and 0.41, respectively.

Urinary 6-sulfatoxymelatonin (aMT6-s) was measured radioimmunologically using kits purchased from IBL Laboratories

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(Hamburg, Germany). The sensitivity and the intra-assay and interassay coefficients of variation were 1.7 ng/mL, 9.8%, and 15.3%, respectively. The value of assay sensitivity was assigned for a woman (n = 1) who had an undetectable level. To adjust for variation in the diluteness of urine, urinary aMT6-s levels were expressed as urine aMT6-s/urine creatinine.

We excluded 31 women from analyses because of incomplete or unreliable responses to the dietary questionnaire (criteria shown in ref. 7). Women who reported having cancer (n = 6) or heart disease (n = 10) were excluded. As exogenous estrogen may suppress melatonin level (9), women using hormone replacement therapy (n = 6) or contraceptive pills (n = 1) were excluded from the study. Because it was not possible to obtain information on the use of diuretics and β-blockers, which can affect urinary aMT6-s levels (10), we further excluded 55 women with the diagnosis of hypertension. The urine samples from 34 women were insufficient for the measurement. Hence, the remaining 289 women were the focus of this report.

Urinary aMT6-s level was transformed into logarithmic values for statistical analysis. Intakes of vegetables and other foods and nutrients were adjusted for total energy after log transformation by using the residual method proposed by Willett (11). The relationship between vegetable intake and urinary aMT6-s level was assessed by linear regression models. Geometric means of urinary aMT6-s levels according to the quartile of vegetable intake were provided using analysis of covariance models. Potential confounders, such as age, body mass index, alcohol intake, menopausal status, and day length (the number of hours of daylight between dawn and dusk), of the day before urine collection were included into models as covariates.

Results

The characteristics of the study subjects were shown in Table 1.

Table 2 shows the geometric means of urinary aMT6-s according to the quartile of vegetable intake. The mean urinary aMT6-s was 15.9% higher in women with the highest quartile of vegetable intake than it was in those with the lowest quartile of intake after controlling for age, total energy, body mass index, smoking status, alcohol intake, menopausal status, and day length. There was a significant trend between urinary aMT6-s and intake of vegetables after controlling for the covariates. A similar tendency was observed for the association of aMT6-s with green and yellow vegetables as well as other vegetables, although the association with other vegetables was of borderline significance.

Table 1. Basic characteristics of 289 women

Variables	
Age (y)	48.1 (8.7)
Body mass index (kg/m^2)	22.8 (2.8)
Alcohol intake (mL/d)	5.4 (13.4)
Dietary intake	
Total energy (kcal/d)	2,252 (782)
Vegetables (g/d)	440 (315)
Green and yellow vegetables (g/d)	162 (151)
Other vegetables (g/d)	279 (180)
Fruits (g/d)	138 (116)
Total protein (g/d)	90.3 (34.8)
Total fat (g/d)	64.5 (27.5)
Carbohydrate (g/d)	318 (110)
Current smokers (%)	2.1
Ex-smokers (%)	7.7
Postmenopausal (%)	39.8
Family history of breast cancer* (%)	3.5

Values are means (SD) or percentage.
*Among first-degree relatives.

Table 2. Geometric means of urinary aMT6-s according to quartile of vegetable intake

Quartile (g)	Median (g)	Urinary aMT6-s (ng/mg creatinine)	
		Age and energy-adjusted	Adjusted*
Vegetables (total)			
Q1 (<272)	223	31.1 (26.7-36.3)	32.1 (28.0-36.8)
Q2 (272-367)	319	31.9 (27.4-37.2)	32.4 (28.3-37.1)
Q3 (368-498)	414	34.2 (29.4-40.0)	34.8 (30.4-39.9)
Q4 (>498)	593	38.8 (33.2-45.4)	37.2 (32.3-42.7)
P_{trend}†		0.02	0.04
Green and yellow vegetables			
Q1 (<83)	68	32.2 (27.6-37.6)	32.7 (28.5-37.6)
Q2 (83-20)	101	30.7 (26.4-35.8)	32.7 (28.5-37.4)
Q3 (120-184)	154	34.1 (29.2-39.7)	32.9 (28.7-37.6)
Q4 (>184)	232	39.1 (33.4-45.7)	38.3 (33.3-44.0)
P_{trend}		0.01	0.04
Other vegetables			
Q1 (<178)	147	29.5 (25.3-34.3)	30.6 (26.7-35.0)
Q2 (178-231)	207	34.6 (30.0-40.3)	34.7 (30.3-39.8)
Q3 (232-313)	271	37.5 (32.2-43.7)	35.9 (31.3-41.2)
Q4 (>313)	374	34.5 (29.6-40.2)	35.2 (30.7-40.4)
P_{trend}		0.06	0.07

*Adjusted for age, total energy, body mass index, alcohol intake, menopausal status, and the day length of the day before urine collection.
†The values for trend were from linear regression models.

Table 3 shows the association of fruit intake with urinary aMT6-s. Fruit intake was positively associated with urinary aMT6-s but this association was not statistically significant. There was no significant association of urinary aMT6 with total energy and other food groups and nutrients, such as grains, potatoes, meats, fishes, dairy foods, protein, fat, and carbohydrate. Carotene, dietary fiber, and vitamins, which are abundant in vegetables, were marginally significantly associated with urinary aMT6-s (data not shown).

As exposure to light at night may affect the urinary aMT6-s levels (12), an additional survey was conducted 3 years after to obtain information on sleeping habits around the time when the urine sampling had been conducted. Out of the 289 women, 204 responded to the second survey. Further adjustment for the frequency of being awake around 1:00 and 2:00 a.m. (the approximate time of the melatonin peak) did not substantially alter the results; the mean urinary aMT6-s was 20.7% higher in women with the highest quartile of vegetable intake than it was in those with the lowest quartile of intake after controlling for the covariates (P_{trend} = 0.05).

When we analyzed data separately for premenopausal and postmenopausal women, the association of vegetable intake with urinary aMT6-s level was weak in postmenopausal women; mean aMT6-s levels in the lowest and the highest quartiles of vegetable intake was 32.8 and 37.3 ng/mg creatinine, respectively, in postmenopausal women. The corresponding values for premenopausal women were 31.9 and 41.0 ng/mg creatinine, respectively.

Table 3. Geometric means of urinary aMT6-s according to quartile of fruit intake

Quartile (g)	Median (g)	Urinary aMT6-s (ng/mg creatinine)	
		Age and energy-adjusted	Adjusted*
Fruits			
Q1 (<69)	47	30.3 (26.0-35.3)	31.9 (27.8-36.6)
Q2 (69-107)	88	33.1 (28.4-38.6)	35.0 (30.6-40.1)
Q3 (108-157)	127	33.2 (28.5-38.7)	31.8 (27.7-36.4)
Q4 (>157)	228	39.6 (33.9-46.2)	37.8 (33.0-43.3)
P_{trend}†		0.04	0.11

*Adjusted for age, total energy, body mass index, alcohol intake, menopausal status, and day length.
†The values for trend were from linear regression models.

Discussion

To our knowledge, this is the first report on vegetable intake and melatonin levels in humans. Vegetable intake was moderately but significantly associated with urinary aMT6-s level. We speculate that this association may be explained by the melatonin contained in vegetables. Hattori et al. (3) reported that feeding chicks a diet containing plant products rich in melatonin increased blood melatonin levels.

Urinary specimens were not collected over a long period (12 or 24 hours). However, the validity of the use of the first-void morning urine has been previously reported. Urinary aMT6-s level in morning urine is strongly correlated with total nocturnal plasma melatonin output and peal nocturnal melatonin value (13). Sufficient reproducibility of measurement of aMT6-s in morning urine over 5 years (intraclass correlation coefficient = 0.58; ref. 14) has been also reported.

The food-frequency questionnaire, like all methods of dietary assessment, is subject to measurement error. Our questionnaire was designed to measure an individual's relative intakes of foods and nutrients rather than absolute values. The data presented for vegetables may have been overestimated because vegetable intake estimated from the questionnaire was 46% higher than that estimated from the 12 daily diet records. Our questionnaire included 13 items for specific vegetables, such as tomato, pumpkin, spinach, Japanese radish, cabbage, carrot, etc. Besides these food items, we took into account some dishes that include vegetables as ingredients, which may have yielded higher intake compared with the diet records. However, it is likely that this measurement error was unrelated to urinary aMT6-s levels and led to an underestimation of the true associations.

As data for melatonin concentration are available only for a few plants, it is not possible to estimate the dietary melatonin intake from vegetables or from the entire diet. Hattori et al. (3) measured the melatonin concentrations in plants commonly consumed as vegetables among the Japanese. In their study, melatonin concentrations ranged from 24.6 pg/g tissue for cucumber to 657.2 pg/g tissue for white radish sprouts. Serum melatonin levels in normal humans are very low during most of the day but increase significantly to a mean of 80 pg/mL (range, 0-200) between 2:00 and 4:00 a.m., and remain elevated during the normal hours of sleep, falling sharply to daytime values around 9:00 a.m. (15). Even 10 μg of melatonin infusion raises serum melatonin concentration ~40 times more at 5 minutes after administration (from 12 ± 5 to 487 ± 377 pg/mL; ref. 16). However, consumption of 400 g of white radish sprouts should provide only 0.3 μg of melatonin. We cannot rule out the possibility that melatonin in vegetables may not be sufficient to affect blood melatonin or urinary aMT6-s levels. In such a case, vegetable intake may be merely a correlate of certain factors, which are associated with urinary aMT6-s.

Cagnacci et al. (17) suggested that the effects of melatonin on some biological functions, such as hypothermic response, are reduced in aged women, which may partially explain the observed weak association of urinary aMT6-s with vegetable intake in postmenopausal women.

Thus far, melatonin has been identified in some grains, nuts, and fruits, but we did not observe significant associations of urinary aMT6-s with intakes of these food groups. In these food groups, the number of foods that contain melatonin may be very limited or the concentration of melatonin may vary greatly in different foods belonging to the same food group. Folate deficiency decreases melatonin secretion in rats (18). Although folate intake itself was nonsignificantly associated with urinary aMT6-s levels (P = 0.10), it is possible that the association of vegetable intake with urinary aMT6-s may be attributable to melatonin together with folate in vegetables. Fruits are also rich in folate. Like vegetables, high intake of fruits has been associated with reduced risks of cancer and cardiovascular disease (1). Our data did not deny the possibility that melatonin contained in fruits may be implicated in these associations.

Clearly, more extensive studies to determine the melatonin concentrations in a wider variety of vegetables, as well as other foods, will be necessary before our results are thoroughly understood. In addition, evidences for the beneficial effects of melatonin on cancer and other diseases is mainly based on laboratory data and must be confirmed in epidemiologic studies. Nonetheless, our findings might stimulate studies investigating the role of dietary melatonin in health.

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Short Communication

Leanness, Smoking, and Enhanced Oxidative DNA Damage

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Abstract

An increased risk of some forms of cancer, including lung cancer, among lean individuals has been consistent; however, there is a paucity of biological evidence supporting this relation. Subjects analyzed were 177 healthy Japanese workers who participated in a lifestyle intervention study. The levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage, were measured using an automated high-pressure liquid chromatography and urinary creatinine levels were adjusted for before statistical analysis. A clear inverse association was found between body mass index (BMI) and 8-OHdG levels among smokers [Pearson correlation coefficient (r) = −0.48], and the association did not materially change after adjustment for potential confounding factors. In contrast, no apparent relation was observed between BMI and 8-OHdG levels among nonsmokers (r = −0.12), although lean nonsmokers had a slightly higher mean of 8-OHdG levels compared with nonlean nonsmokers. The interaction of smoking and BMI reached statistical significance (P = 0.04). Leanness may enhance oxidative DNA damage induced by smoking and thus serve as a marker of host susceptibility to smoking-related cancers. (Cancer Epidemiol Biomarkers Prev 2006;15(3):582–5)

Introduction

Obesity has been admitted as a risk factor of cancer (1); however, little attention has been paid to the role of leanness in carcinogenesis. Epidemiologic studies have shown an inverse association between body mass index (BMI) and total cancer risk (2) or the risk of several cancer forms, including cancer of the lungs (3) and esophagus (4). Several studies (2-4) reported a stronger association among smokers than among nonsmokers. Leanness is thus hypothesized to represent a host susceptibility to these smoking-related cancers. However, controversy continues regarding causal role of leanness in carcinogenesis due to a potential bias in epidemiologic studies (5). For instance, the effect of preclinical cancer on weight loss cannot be completely ruled out. Moreover, because smoking is related to lower BMI levels (6), the inverse association between BMI and cancer risk may merely reflect smoking-induced weight loss.

Oxidative DNA stress is thought to play a major role in carcinogenesis (7), and increased levels of 8-hydroxydeoxyguanosine (8-OHdG), a reliable marker of oxidative DNA damage, have been detected in urine of smokers (8, 9) or in lung cancer tissue (10). However, the association between BMI and urinary 8-OHdG levels has been inconsistent; two studies (8, 9) reported an inverse association, whereas others (11, 12) failed to detect such association. Moreover, there is limited evidence suggesting a modifying effect of smoking on BMI and 8-OHdG levels (8). We therefore investigated whether leanness modulates the relation of smoking to oxidative DNA damage among healthy working employees, using an automated high-pressure liquid chromatography (HPLC; ref. 13).

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Materials and Methods

Data were obtained from the baseline survey of a worksite lifestyle intervention study, in which 179 volunteers ages 28 to 57 years of a Japanese city office participated. A written informed consent was obtained. The study protocol has been approved by the ethics committee of Kyushu University.

Health-related lifestyles were ascertained using a detailed questionnaire. Ever smokers were defined as those who smoked 100 cigarettes or more in their lifetime. Current smokers consuming cigarettes on a daily basis were asked about cigarette consumption a day, whereas current smokers consuming less than daily basis were defined as occasional smokers. Regular alcohol drinkers were defined as those who consumed alcohol beverage on a weekly basis over the recent 1-month period, and they were asked about the frequency and quantity per occasion of consumption for each type of five alcohol beverages—shochu, beer, sake, wine, and whisky/liquor. Those who engaged in any leisure-time physical activities during the past month were asked about the names, frequencies, and minutes or hours engaged per occasion of each activity.

Casual urine samples, collected mostly between 5 to 6 p.m. before super, were kept in tubes stored in a cooler box overnight and then frozen at −80°C until analysis. Urinary samples were analyzed for 8-OHdG using an automated HPLC system composed of two columns and an electrochemical detector (13). In short, the urinary 8-OHdG level was determined using an apparatus in which the pump 1 (Shiseido Nanospace SI-2), the sampling injector (Gilson 231XL), the guard column for the HPLC-1 (valve 1, pump 3), the HPLC-1 column, the UV detector (Toso UV-8020, microcell), the HPLC-2 column (valve 2, loop, pump 2), and the EC detector (ESA Coulochem 2) were connected. Urine samples were defrosted and 50 μL of each was mixed with the same volume of a dilution solution containing the ribonucleoside marker 8-hydroxyguanosine (120 μg/mL) and 4% acetonitrile in a solution of 130 mmol/L sodium acetate (pH 4.5) and 0.6 mmol/L H_2SO_4. The urine solutions were centrifuged at 13,000 rpm for 5 minutes. A 20 μL aliquot of each supernatant was injected into the first HPLC (MCI

GEL CA08F, 7 μm, 1.5 × 150 mm, 2% acetonitrile in 0.3 mmol/L sulfuric acid, 37 μL/min) from the sampling injector, via the guard column, and the chromatogram was recorded by the UV detector (254 nm). A aliquot of the fraction containing 8-OHdG was automatically injected into the second HPLC column [Shiseido, Capcell Park C18, 5 μm, 4.6 × 250 mm, 10 mmol/L sodium phosphate buffer (pH 6.7), 5% methanol, plus an antiseptic Reagent MB (100 μL/L), 1 mL/min]. Finally, the 8-OHdG was detected by an EC detector with a guard cell (5020) and an analytic cell (5011). The accuracy of the measurement, estimated from the recovery of an added 8-OHdG standard, was 90% to 98%. When the same urine sample was analyzed thrice, the variation of the data was within 7%. Urinary creatinine levels of the same urine sample were simultaneously measured by using anion exchange chromatography in the HPLC-1 step.

8-OHdG levels were adjusted for urinary creatinine levels and then log-transformed before analysis. Subjects were divided into either nonsmokers, including past smokers, or current smokers, including occasional smokers. Weight was measured in a light cloth and information about height was obtained from record of the latest checkup. BMI was calculated as body weight in kilograms divided by the square of height in meters. Ethanol consumption was estimated by multiplying the frequency of consumption and amount consumed per occasion for each of five alcohol beverages and summed. Intensity of each leisure-time physical activity was determined in terms of metabolic equivalent (MET) according to the literature (14). MET hours per week for a specific activity was calculated by multiplying weekly hours spent in that activity and the corresponding intensity, and weekly MET hours of total activity was estimated by summing the MET hours for each type of activity. The association between BMI and 8-OHdG levels was assessed by Pearson correlation coefficient (r). Multiple regression and analysis of covariance were used to estimate regression coefficients and means, respectively, while adjusting for sex, age (continuous), alcohol consumption (<3.0, 3.0-22.9, or ≥23.0 g/d), and physical activities (<1.0, 1.0-9.9, or ≥10.0 MET-h/wk). Effect modification was tested by adding a cross-product term of smoking status and BMI (continuous) in the model. Preliminary analysis indicated that data for two smokers who had extremely high BMI (over the mean value plus 3 SD) were outliers, and thus these were excluded. All statistical tests were two-tailed and were considered to be statistically significant at the 0.05 level. All analyses were done with SAS (15).

Results

Characteristics of the study subjects were shown in Table 1. Of 177 subjects, 38 (21%) were female and 49 (28%) were current smokers. Only three women were current smokers, and they were all occasional smokers. Among men, means of BMI were 24.7 and 24.3 kg/m^2 for smokers and nonsmokers, respectively. BMI was not significantly associated with the number of cigarette consumption among daily smokers (r = 0.11).

The levels of 8-OHdG ranged from 1.2 to 11.4 μg/g creatinine, with median of 3.9 μg/g creatinine. The geometric means of 8-OHdG levels were 3.70, 4.50, and 4.52 μg/g creatinine for nonsmokers, occasional smokers, and daily smokers, respectively. A weak positive association was observed among daily smokers between the number of cigarette smoked a day and 8-OHdG levels (r = 0.21). Among nonsmokers, geometric means of 8-OHdG levels were 3.68 and 3.74 μg/g creatinine for men and women, respectively.

As shown in Fig. 1, a clear inverse association emerged among 49 current smokers (r = −0.48; P = 0.0004). Adjustment for daily cigarette consumption, with 0.5 assigned to occasional smokers, slightly strengthened the association (partial r = −0.51), whereas analysis excluding occasional smokers somewhat attenuated the association (r = −0.41). Regression coefficient of log-transformed 8-OHdG on BMI (−0.069 μg/g creatinine per unit BMI) did not materially change after adjustment for age, sex, alcohol consumption, and physical activities (−0.070 μg/g creatinine per unit BMI). In contrast, 8-OHdG levels did not apparently correlate with BMI among 128 nonsmokers (r = −0.12; P = 0.18), although a marginally significant inverse correlation was observed among nonsmoking men (r = −0.19; P = 0.06).

Table 1. Characteristics of study subjects by gender

	Men (n = 139)	Women (n = 38)
Age (y)	42 (7)	39 (8)
Current smokers (%)	33	8
Occasional smokers among current smokers (%)	13	100
No. cigarettes smoked a day among daily smokers	19 (10)	—
Past smokers among nonsmokers (%)	40	3
Height (cm)	171 (5)	159 (5)
Weight (kg)	71 (9)	54 (7)
Body mass index (kg/m^2)	24.4 (2.8)	21.5 (2.4)

NOTE: Values were mean and standard deviation (in parenthesis) unless otherwise stated.

Subjects were divided into six groups by smoking status and BMI category (tertiles of BMI among smokers: <23.1, 23.1-25.1, and ≥25.2 kg/m^2). Multivariate adjusted mean of 8-OHdG levels was statistically significantly higher among smokers than among nonsmokers in the lowest tertile of BMI (geometric mean: 5.22 μg/g creatinine for smokers versus 4.03 μg/g creatinine for nonsmokers; P = 0.03), whereas 8-OHdG levels did not materially differ according to smoking status in the highest tertile of BMI (geometric mean: 3.56 μg/g creatinine for smokers versus 3.56 for nonsmokers μg/g creatinine; P = 0.90). Among nonsmokers, mean of 8-OHdG levels in the lowest tertile of BMI was slightly higher than those in upper categories of BMI (geometric mean: 4.03, 3.47, and 3.50 μg/g creatinine for the lowest, medium, and highest tertile, respectively). The interaction of smoking and BMI (continuous) reached statistical significance ($P_{interaction}$ = 0.04).

Discussion

The source of urinary 8-OHdG may be the hydrolysis of 8-OH-dGTP by the nucleotide sanitization enzyme MTH1, the mucleotide excision repair of DNA, and the apoptosis of oxidatively damaged cells (16, 17). Urinary excretion of 8-OHdG is a useful biomarker reflecting general average risk of a promutagenic oxidative adduct in DNA, and thus carcinogenesis of all tissues and organs (16). Using an automated HPLC method, we investigated the association of smoking, BMI, and levels of urinary 8-OHdG among a healthy working population and found a clear inverse association between BMI and urinary 8-OHdG levels among smokers.

Most HPLC methods developed thus far have not been suitable for the analysis of 8-OHdG in epidemiologic studies because of complicated manual procedures involved (reviewed in ref. 13). ELISA method is simple and cost-efficient, but it produced two to four times higher values compared with those obtained using HPLC, probably due to cross-reactions to substances having similar structure to 8-OHdG (18). The method we used is able to analyze large samples with reasonable reproducibility (13). In addition, the urinary 8-OHdG level was unchanged, even when urine samples were kept at room temperature for 24 hours (9).